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5 A MINISWINE MODEL OF HEATSTROKE

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Running Head: Heatstroke in Miniswine

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ABSTRACT

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38 We developed a miniswine model of passive heatstroke, in part, to explain
39 the variable hyper-, normo- and hypokalemia seen in heatstroke victims. After a
40 baseline period ($T_{amb}=26-27^{\circ}\text{C}$), anesthetized and instrumented miniswine ($n=13$,
41 mass=44.6 kg) were ramped to $41-43^{\circ}\text{C}$, 60% RH; 13 controls were treated
42 identically, but T_{re} was maintained at 38°C . T_{re} of the experimental miniswine rose
43 nearly linearly to $45-46^{\circ}\text{C}$ until death (approx. 4h). The response patterns of mean
44 arterial pressure, heart rate, plasma K^{+} , LPS, Ca^{++} , inorganic phosphate, lactate
45 and a variety of other clinical chemical and physiological variables were determined.
46 An explanation for the variability of plasma K^{+} in heatstroke victims was proposed.
47 This model may be useful in characterizing the multisystemic pathology of severe
48 heat injury and be useful for assessing innovative therapeutic regimens.

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INDEX TERMS:

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Potassium, Calcium, Phosphate, pH, Hyperthermia, LPS, Heatstroke, Swine

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Disclaimers

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The views, opinions, and/or findings contained in this report are those of the authors and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other official documentation.

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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

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INTRODUCTION

Heatstroke is a medical emergency requiring immediate intervention to prevent death. Alterations in plasma potassium ion concentrations change membrane potentials, influence contractility of muscle and may be an important component of the pathophysiology of heatstroke. While plasma K^+ rises during severe exercise, (Knochel, 1990; Knochel, 1992; Knochel, 1996) there are controversies in the literature as to whether the plasma concentrations of K^+ , Ca^{++} and inorganic phosphate (Pi) rise, fall or remain unchanged as a result of heatstroke (Ayus & Arieff, 1990; Baxter & Teschan, 1958; Hubbard, 1979; Khogali & Mustafa, 1987; Knochel, 1992; Shapiro & Cristal, 1987) and whether respiratory alkalosis develops prior to metabolic acidosis in heatstroke victims (Boyd & Beller, 1975; Hart *et al.*, 1982; Shibolet *et al.*, 1967).

Heatstroke victims show clinical symptoms resembling those of gram negative sepsis, including circulatory shock, elevated plasma levels of gram negative bacterial lipopolysaccharides (LPS) and cytokines and disseminated intravascular coagulation (Bouchama *et al.*, 1993; Bouchama *et al.*, 1991; Gathiram *et al.*, 1988; Graber *et al.*, 1971). We have previously seen in nonhuman primates during experimental heatstroke that LPS enters the circulation at 42.5-43°C (Gathiram *et al.*, 1987a; Gathiram *et al.*, 1988), probably as a result of two factors: a) a reduced splanchnic blood flow caused by hyperthermia led to ischemic damage to the intestinal wall and leakage of LPS from the lumen (where it is always present) into the circulation at T_c up to 43.5°C (Proppe, 1980), and b) at higher temperatures, direct thermal damage to the gut wall and other tissues (Gathiram *et al.*, 1987b).

96 This paper describes an experimental model of heatstroke in an animal
97 model with a cardiovascular system similar to that of humans and of sufficient
98 volume to permit serial blood samples and the detection of circulating
99 lipopolysaccharides. (Gathiram *et al.*, 1987c; Gathiram *et al.*, 1988). Miniswine
100 physiology is close to that of humans, is virtually identical biochemically and
101 physiologically to standard swine, and this model has the extremely important
102 advantage of being of manageable adult size (Hannon *et al.*, 1990). We have used
103 this model previously to study exertional hyperthermia (Gentile *et al.*, 1996) and the
104 current study was designed to evaluate its usefulness as a model for passively-
105 induced heatstroke.

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MATERIALS AND METHODS

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The experimental protocol was approved by the local Institute Animal Care and Use Committee of U.S.A.R.I.E.M. and guidelines regarding humane use of animals were rigorously applied. Miniswine were obtained from Charles River Laboratories and housed and fed in the AAALAC accredited facility at USARIEM for at least one month before use. At the time of the experiment the mean body masses were 44.6 ± 5.6 Kg and mean ages were 14.3 ± 1.70 months. They were fed daily with standard minipig chow, had water ad lib, and were inspected regularly by the veterinary staff to ensure healthy status. Fecal flotation examination was made to document the absence of endoparasites.

Anesthesia and Surgery. Isoflurane in 100% oxygen was used at a concentration of 4-5% for induction, followed by 2.5-3% for maintenance. Surgical procedures were carried out under general anesthesia without mechanical ventilation, in a temperature-controlled chamber. A catheter was placed into the

122 right femoral artery for blood sampling, monitoring blood pressure and the
123 parenteral administration of saline and drugs. If rectal temperature (T_{re}) fell during
124 these procedures, the pig was warmed passively to a rectal temperature of
125 $38 \pm 0.1^\circ\text{C}$. The effect of isoflurane on the cardiovascular system is believed to be
126 limited, and therefore its effect on heat loss from hyperthermia is expected to be
127 small.

128 *Heat Stress.* After baseline measurements were taken for 60 minutes ($n=13$),
129 the environmental temperature (T_{env}) within the chamber was adjusted to $41-43^\circ\text{C}$
130 and 60% relative humidity for 3-5 hours. From time zero, blood samples (5 ml) were
131 taken every 20 min by sterile technique after removing the "dead volume" in
132 catheter lines. After sampling, the dead volume of blood was then injected back
133 through the catheter into the miniswine, followed by 2 ml of sterile saline. Control
134 animals ($n=13$) were treated the same except that T_{env} was not raised, and T_{re}
135 was maintained at 38°C . The control animals were not allowed to survive and were
136 euthanized with pentobarbital according to AVMA recommendations. The
137 experimental animals were necropsied (AVMA, 1986).

138 *Blood Samples.* For gas determinations, 0.8 ml of blood was collected into 1
139 ml syringes which had been flushed with heparin, sealed, cooled rapidly on ice and
140 analyzed within 10 minutes. For serum, blood was permitted to clot for 30 min at
141 room temperature, and then centrifuged on a clinical centrifuge for 10 min at 4°C .
142 For plasma LPS determinations, blood was collected into EDTA tubes, placed on
143 melting ice for up to 20 minutes, and then centrifuged for 8 min at $1,500 \times g$ at 0°C .
144 Subsequently, the plasma was removed under sterile conditions in a laminar flow
145 hood and processed immediately. Plasma (150 μl , 0°C) was added to 300 μl of
146 1.88% perchloric acid at 20°C , briefly vortexed, and incubated for 20 min at 37°C . It
147 was then neutralized with 87 μl of 0.5N NaOH, vortexed 10 sec, and frozen. The

148 next day it was thawed and centrifuged for 15 min at 3,000 x g at 22°C. An aliquot
149 (50 ul) was added by sterile technique to the wells of a microtiter plate and LPS
150 determined by a recent modification of the colorimetric methodology of the Limulus
151 amebocyte lysate technique (Associates of Cape Cod, Falmouth MA).

152 *Laboratory Analyses.* Blood gases, pH and hemoglobin were determined on
153 an AVL 995Hb blood gas analyzer, which automatically corrected for temperature
154 up to 44°C. For $T_{re} > 44^{\circ}\text{C}$, required temperature corrections of pH, PCO_2 , and PO_2
155 were calculated according to manufacturer's instructions. Hematocrit was
156 determined after centrifugation of blood on a clinical hematocrit centrifuge. Total
157 protein was determined by refractometry with an AOTS refractometer (AO Scientific
158 Instruments). Calcium, magnesium, inorganic phosphate, creatinine, BUN, glucose,
159 lactate and enzymes were determined on a Ciba Corning Express 500 clinical
160 analyzer. Sodium and potassium were determined on an IL943 flame photometer.

161 *Statistics.* In order to account for inter-individual variability and, in some
162 cases, small changes in several of the measured parameters from the baseline
163 period, data were converted to changes or percent changes from baseline values,
164 and then analyzed by ANOVA and the Tukey post-hoc test. Absolute results are
165 expressed as mean \pm s.d., and the null hypothesis was rejected at $p < 0.05$.

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167 RESULTS

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169 Following a short lag time, and during the remainder of the heating period,
170 T_{re} rose at a constant rate of $1.48^{\circ}\text{C}/\text{hour}$ until death which occurred at $45.6^{\circ}\text{C} \pm$
171 0.5°C (not shown).

172 *Cardiovascular Parameters.* Upon heating, the heart rate (HR) rose
173 gradually from a baseline of 116 beats per min (bpm) to a plateau of 130 bpm at T_{re}

174 between 38-43°C, then rose rapidly to a peak of over 175 bpm at Tre=44 - 45°C,
175 followed by a sharp decline to death (Figure 1). Mean arterial pressure (MAP)
176 showed no significant change with heating until Tre reached 42°C and then declined
177 until death, with a shoulder at 44-45°C, corresponding to the HR peak. However,
178 when the MAP of 3 animals was compared to its own baseline levels, the MAP rose
179 significantly (ca. 20%) upon heating, and then declined as at Tre = 42°C, as
180 occurred in other experimental animals.

181 *Blood Gases, pH and Metabolism.* As shown in Figure 2, the respiratory rate
182 remained stable until approximately 41°C, rapidly rose to a peak at 42°C and then
183 declined until death. PCO₂ also remained stable until Tre reached 41°C, then
184 declined slightly to Tre=43°C, and then rose rapidly to more than 400% of baseline
185 at death. Arterial pH remained at 7.45 until Tre=42°C, then became slightly more
186 alkaline, peaked at 43°C, and then declined rapidly to 6.8-6.9 at death.

187 The initial PO₂ was 480mm Hg due to the inhalation of 97% oxygen (Fig. 3).
188 PO₂ declined slowly as Tre rose, and at 43°C fell rapidly to 200 mmHg at death.
189 Lactate concentration, however, began to rise at 42.5-43°C, despite the high PO₂ at
190 these temperatures.

191 *LPS.* LPS concentrations remained at baseline levels until 43.5°C and then
192 rose significantly (Figure 4).

193 *Blood Chemistry.* Table I records the general trends among these clinical
194 chemical indices of heat injury. The most interesting and prominent effects of heat
195 stress on blood chemistry were seen in K⁺, Ca²⁺, and phosphate (Pi) (Figure 5).
196 As Tre increased, K⁺ rose continuously to values 170-180% of baseline (p<0.001),
197 while Ca²⁺ and Pi gradually fell to minima at 43.5 and 44.5°C, respectively, and
198 then rapidly rose to near baseline values at death.

225 accredited facilities, fed laboratory quality chow, and ventilated with filtered air, while
226 the monkeys were maintained under less clean conditions in family groups, fed
227 fresh fruit and vegetables, and breathed unfiltered air. Since: (1) LPS is present
228 during heatstroke in miniswine, monkeys, and in some clinical studies, (2) anti-LPS
229 antibodies and prior infections protected against heatstroke in monkeys (DuBoise *et al.*,
230 1983; Gathiram *et al.*, 1987b; Gorman & Proppe, 1984), and (3) heat stress
231 protected against LPS challenge (Ryan, 1993; Ryan *et al.*, 1992), it is reasonable to
232 conclude that LPS and cytokines, (Bouchama *et al.*, 1993; Bouchama *et al.*, 1991)
233 may participate in the pathophysiology of heatstroke. Therapeutic interventions for
234 heatstroke therefore should consider anti-LPS and anti-cytokine procedures.

235

236 K⁺. Although severe exercise often led to hyperkalemia (Sjogaard, 1990), the
237 situation in heatstroke is less predictable. Some heatstroke studies reported
238 hyperkalemia in humans and experimental animals, (Gisolfi *et al.*, 1991; Hubbard,
239 1979; Hubbard, 1990a; Khogali *et al.*, 1983; Knochel, 1992; Lundvall, 1972;
240 Pettigrew *et al.*, 1974) while others reported hypokalemia (Baxter & Teschan, 1958;
241 Khogali & Mustafa, 1987). This miniswine study shows that hyperkalemia is the
242 *primary* response to heat stress, as previously seen in the rat (Gisolfi *et al.*, 1991).
243 In order to propose a mechanism to explain the reports of normokalemia and even
244 hypokalemia of heatstroke, we note the following in studies on cultured cells:

245 (1) Heating accelerates Na⁺ influx by diffusion and speeds its efflux by the
246 Na⁺K⁺ATPase pump, but with a net increase in [Na⁺]. (Boonstra, 1984; Bowers Jr *et al.*,
247 1984; Ruifrok *et al.*, 1985; Ruifrok *et al.*, 1986; Yi, 1979)

248 (2) However, heating does not markedly increase outward diffusion of K⁺.
249 (Willis & Anderson, 1997)

250 (3) Rather, heat activates the normally inactive K,Cl cotransporter in the
251 plasma membrane, leading to a net loss of intracellular K^+ to the interstitial fluid and
252 the circulation. (Willis & Anderson, 1997)

253 Generally, hyperthermia causes K^+ to leave cells by both the K,Cl cotransporter and
254 by diffusion through leak channels into interstitial fluid faster than its reuptake by the
255 Na^+K^+ ATPase pump. When these observations on whole cells are considered with
256 the hyperkalemia and renal failure seen here in miniswine, the following model may
257 partially explain the variability in plasma K^+ reported for heatstroke victims:

258 (1) During initial hyperthermia, the kidney excretes the elevated plasma K^+
259 into the urine, with additional losses through secretions such as sweat and saliva.
260 However, eventually, the combination of more severe hyperthermia, elevated heart
261 rate, and, perhaps, hypovolemia leads to a drop in blood pressure (Figure 1), which,
262 together with mineralocorticoid secretion, reduces kidney function, (Figure 6) and
263 leads to *primary hyperkalemia* in the heat illness victim (Morimoto, 1987).
264 Furthermore, if hyperthermia should cause some rhabdomyolysis, then K^+ from
265 those cells would also contribute to the hyperkalemia. (Knochel, 1990)

266 (2) When the patient is treated with an infusion of electrolyte solution, blood
267 pressure would be expected to rise with a partial or complete recovery of renal
268 function, as has been seen following the rehydration of dehydrated rats (Morimoto,
269 1987). This would cause a net excretion of apparently "excess" K^+ in the plasma
270 and lead to *normokalemia*.

271 (3) Eventually, cooling procedures would lower core temperatures, which
272 would reduce and eventually stop the activity of the K,Cl cotransporter and the loss
273 of K^+ from cells. The cells, however, now would "sense" a net loss of K^+ from the
274 cytoplasm and operate K^+ membrane transporters at high rates to restore K^+ to
275 normal values by pumping it from the plasma, and by so doing, lead to a *secondary*

276 *hypokalemia*. Thus, depending upon the timing of blood samples one could
277 theoretically record hyperkalemia, normokalemia or hypokalemia from a single
278 heatstroke victim. Additionally, hypokalemia could occur from diets containing
279 insufficient K^+ , and from K^+ losses due to prolonged sweating.

280 The consistent rise in plasma K^+ could depolarize membrane potentials, lead
281 to depressed muscle contractility, alter neurotransmitter release at synapses
282 (Cochran, 1995), and have other physiological consequences (Andreoli *et al.*, 1990;
283 Knochel, 1992). Furthermore, a K^+ concentration of *ca.* 8 mEq/l as seen in this
284 model may represent an *actual* rise to 16 mEq/l in interstitial fluid (Kjellmer, 1961)
285 which could directly effect ventricular fibrillation and cardiac arrest (Bynum *et al.*,
286 1977).

287 Patterns of Response to Heat Stress Like humans, not all miniswine showed
288 identical **patterns** in their physiological and biochemical responses to heatstroke.
289 The most variable responses were noted among those that showed the most
290 significant changes: HR, glucose, and pH (Table 1) In accordance with
291 physiological principles, PCO_2 appeared to fall and pH rose when the respiratory
292 rate rose substantially. Likewise, when respiratory rate fell, the PCO_2 rose and the
293 pH fell.

294 In humans, certain serum enzymes (creatine kinase, AST, aldolase, LDH,
295 and alkaline phosphatase) rise within 5 min of finishing a marathon race (Lijnen *et*
296 *al.*, 1988), and the cellular injury indicated by these elevations is clearly reversible.
297 Paradoxically, animals heated in the experiment for longer than the duration of a
298 marathon raced showed *lower* or no increases in serum enzymes. Therefore, either
299 a) cellular injury in this heatstroke model is present but takes longer to manifest
300 itself (e.g., induction of cytokines?), b) the injury is present and non-reversible, but a
301 rise in enzyme concentrations is precluded by early death from other causes, (i.e.,

302 respiratory depression, acidosis, hyperkalemia, etc.), or c) the cells are *not* severely
303 injured and presumably could be restored to normal function with aggressive
304 intervention. In accordance with this last concept, moderate heating reversibly
305 increases intracellular sodium and calcium ion concentrations in cultured cells
306 (Kiang *et al.*, 1992), but when $T_{amb} > 43^{\circ}\text{C}$, then the rises become irreversible.
307 (Gaffin *et al.*, 1996; Koratich *et al.*, 1997)

308 Despite breathing supplemental oxygen, hyperpnea, which was probably
309 thermally driven occurred in these miniswine. This led to an early alkalosis that
310 repressed respiration (50%) at pH 7.46, in accordance with physiological principles.
311 The metabolic acidosis observed later can lead to reduced contractility of skeletal
312 and cardiac muscles (Blanchard & Solaro, 1984; El-Saleh & Solaro, 1988).
313 Elevations in magnesium concentration oppose those changes and might be
314 considered for experimental use as potential therapeutic agent (Blanchard & Solaro,
315 1984).

316 Our observations on the biphasic alterations in calcium and phosphate
317 concentrations in the miniswine model may explain the previous conflicting
318 observations in heatstroke patients (Shapiro & Cristal, 1987; Shibolet *et al.*, 1976).
319 We found that early hypophosphatemia and hypocalcemia were followed by a late
320 return to control or even overshoot values, possibly explaining the conflicting results
321 in the literature.

322 Plasma lactate rose long before PO_2 fell, suggesting (Hubbard, 1990b) that
323 glycolysis may be elicited by mechanisms other than insufficient oxygen. It is still
324 possible, however, that despite the presence of adequate oxygen in arterial blood,
325 vasoconstriction caused by A-V shunting around an ischemic core led to the
326 elevated lactate production. However, this hypothesis remains to be tested.

327 Most of the prominent changes in physiological and biochemical variables
328 occurred at temperatures above 41°C. Therefore, previous explanations for the
329 pathophysiology of heatstroke based on extrapolations from 39.5-40°C may not be
330 relevant. It is interesting to note that the decline in pH occurred close to the Tre
331 (43.0-43.5°C) at which the marked rises in PCO₂ and LPS occurred. Therefore,
332 blood pH may be a useful predictor of the extent of pathology to be expected from a
333 serious heatstroke episode.

334

335 Alterations in plasma K⁺, PCO₂, pH and LPS in this miniswine model were
336 both temperature- and time-dependent and may be important components of the
337 pathophysiology of heatstroke. Pharmacological agents that reduce cellular
338 potassium leakage or increase Na⁺/K⁺-pump activity and anti-LPS agents should
339 be considered as possible therapy or prophylaxis.

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LEGENDS

Figure 1. Effect of Hyperthermia on Mean Arterial Pressure (MAP) and Heart Rate (HR) in Anesthetized Miniswine. After 1-hr baseline, environmental temperature was raised to 42-43°C. * = $p < 0.05$ compared to controls; # = $p < 0.05$ compared to own baseline values.

Figure 2. Effect of Hyperthermia on Respiratory Rate (Resp Rate), PCO_2 and Arterial pH in Anesthetized Miniswine. Changes from baseline levels. * = $p < 0.05$ compared to controls; # = $p < 0.05$ compared to own baseline values.

Figure 3. Effect of Hyperthermia on PO_2 and Lactate Concentration in Anesthetized Miniswine. Percent change from baseline level. * = $p < 0.05$ compared to controls; # = $p < 0.05$ compared to own baseline values.

Figure 4. Effect of Hyperthermia on Arterial Lipopolysaccharide Concentration in Anesthetized Miniswine. Change from baseline level. * = $p < 0.05$ compared to controls; # = $p < 0.05$ compared to own baseline values.

Figure 5. Effect of Hyperthermia on Arterial Potassium, Calcium and Inorganic Phosphate Concentrations in Anesthetized Miniswine. Percent baseline levels. * = $p < 0.05$ compared to controls; # = $p < 0.05$ compared to own baseline values.

Figure 6. Effect of Hyperthermia on Renal Function. Percent baseline level. * = $p < 0.05$ compared to controls; # = $p < 0.05$ compared to own baseline values.

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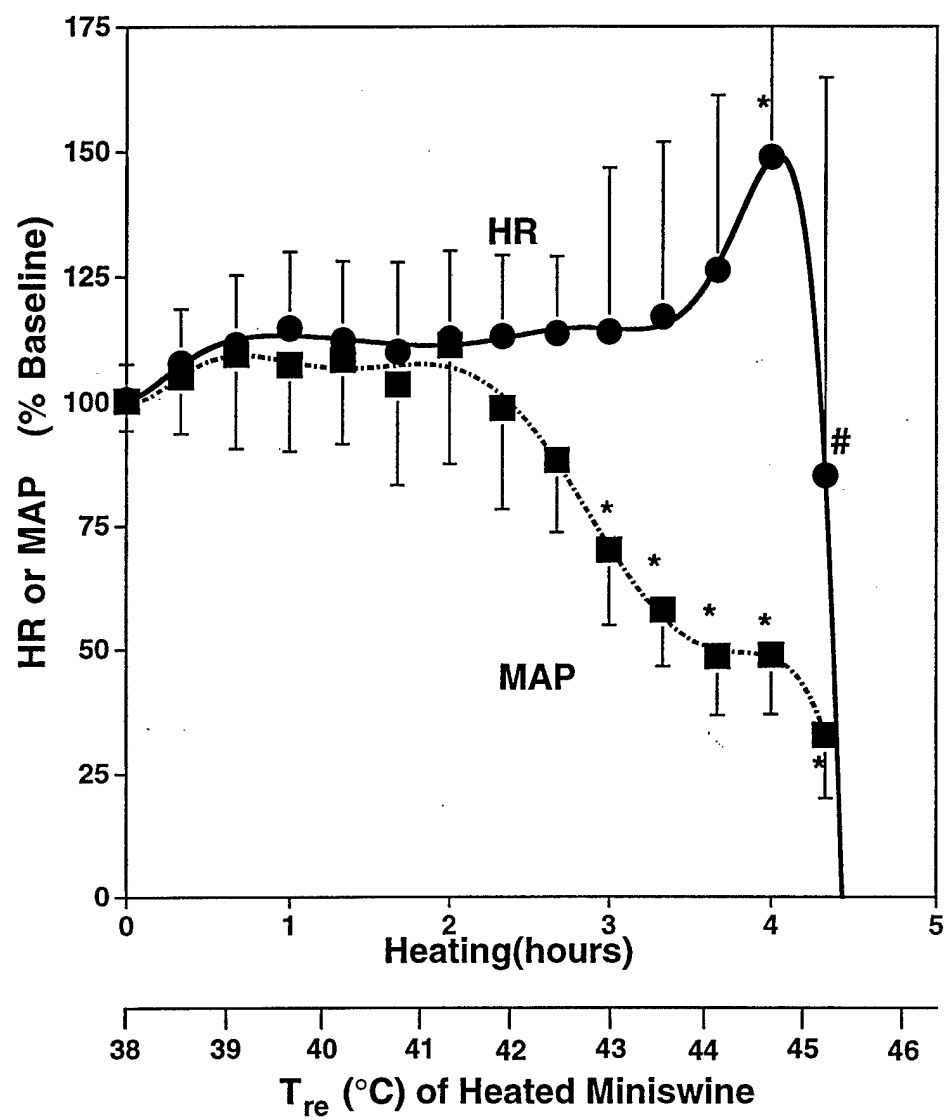
531 **Figure 7.** Model of Potassium Concentration Changes. 1) Under normothermic
532 conditions the Na^+K^+ ATPase membrane pump helps balance K^+ lost into the plasma
533 through K^+ “leak” channels. Heat activates the K,Cl cotransporter (Willis &
534 Anderson, 1997) leading to a net loss of K^+ from cells into the interstitial fluid, which
535 eventually enters the plasma, is filtered into the kidney, and excreted in the urine.
536 2) During severe hyperthermia, as seen in the miniswine model, when hypotension
537 commences, kidney function declines, K^+ excretion is reduced, and leads to a
538 reversible *primary* hyperkalemia. 3) In a heatstroke victim, upon infusion of liters of
539 solution, normovolemia returns, blood pressure rises, and renal function returns to
540 normal, including rapid excretion of the apparently “excess” plasma K^+ lowering the
541 concentration to *normokalemia*. 4) During the cooling process, enabled by elevated
542 sweat rates plus active cooling, the K,Cl cotransporter would be inactivated, and K^+
543 would leave the cell at reduced rates. The cells, however, would “sense” their
544 reduced concentrations of K^+ , and actively transport K^+ from the plasma, leading to a
545 *secondary hypokalemia*.

546

TABLE I. Physiological and Blood Parameters of Anesthetized Heatstroked Miniswine

Parameter	Baseline Values (\pm S.D.)	n/N*	Primary Pattern During Heatstroke	Refs. to Similar Patterns in Other Mammals.
MAP	73.5 (15.8) mm Hg	12/13	Stable or rise, then decline with shoulder at 44°C	(Kregel <i>et al.</i> , 1988)
HR	103.4 (23.2) bpm	8/13	Plateau at 40-43°C then rapid rise to peak and rapid fall	(Kregel <i>et al.</i> , 1988)
Na ⁺	139.4 (3.86) mmol/l	12/13	Small decline, then rapid rise shortly before death	
K ⁺	3.92 (0.30) mmol/l	13/13	Continuous rise to high values.	(Knochel, 1992)
Ca ²⁺	9.39 (0.73) mg/dl	12/13	Small decline then small rapid rise.	(Shapiro & Cristal, 1987; Shibolet <i>et al.</i> , 1976)
LPS	1.12 (0.98) EU/ml	6/7	Stable, then rises rapidly before death	
Mg ²⁺	1.94 (0.44) mEq/dl	4/13	No change	
Pi	6.4 (0.76) mg/dl	7/13	Gradual fall, then rise before the Ca ⁺⁺ rise	
Hct	27.8 (1.36) %	12/13	Continuously rising value	
Hb	8.98 (1.09) g/dl	13/13	Continuously rising value	
PCO ₂	45.74 (5.37) mm Hg	12/13	Stable, small decline then rapid rise.	
pH	7.42 (0.05)	9/13	Stable, small rise after resp peak, then large fall	
Total Protein	6.27 (0.32) g/dl	8/13	Stable, then small rise before death	
Albumin	3.90 (0.23) g/dl	13/13	No change	
Osmolality	290.4 (3.7) mOsm/kg	12/13	Stable, then gradual rise before death	
Glucose	88.0 (20.6) mg/dl	8/13	Slow rise, then fall before death	
Lactate	16.3 (4.06) mmol/l	11/13	Gradual rise to a plateau, or peaks then falls	
Insulin	8.15 (1.39) u/ml	6/6	Gradual rise to occasionally high levels before death	
BUN/Creatinine	18.5 (5.99)	12/13	Stable to 41°C, then slow fall	(Van der Linde <i>et al.</i> , 1992)
AST	29.4 (7.54) u/ml	9/9	Gradual rise	(Van der Linde <i>et al.</i> , 1992)
LDH	299.8 (69.3) u/ml	13/13	Stable, then rise shortly before death	(Van der Linde <i>et al.</i> , 1992)
ALT	45.2 u/ml	13/13	No change	(Van der Linde <i>et al.</i> , 1992)
CK	197.3 (31.3) u/ml	13/13	No change	(Van der Linde <i>et al.</i> , 1992)

* Number of experimental animals actually showing the pattern / Total tested



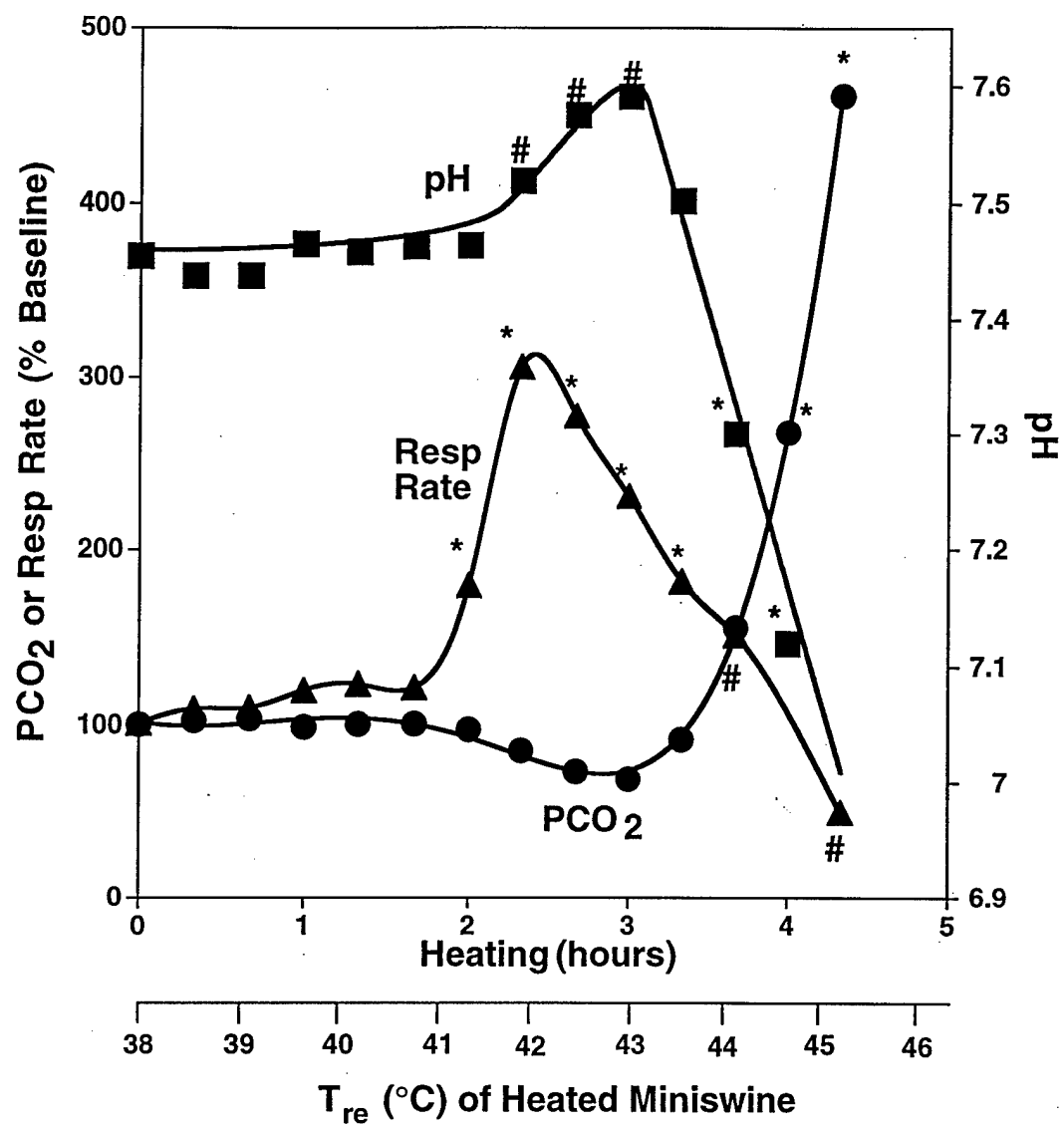
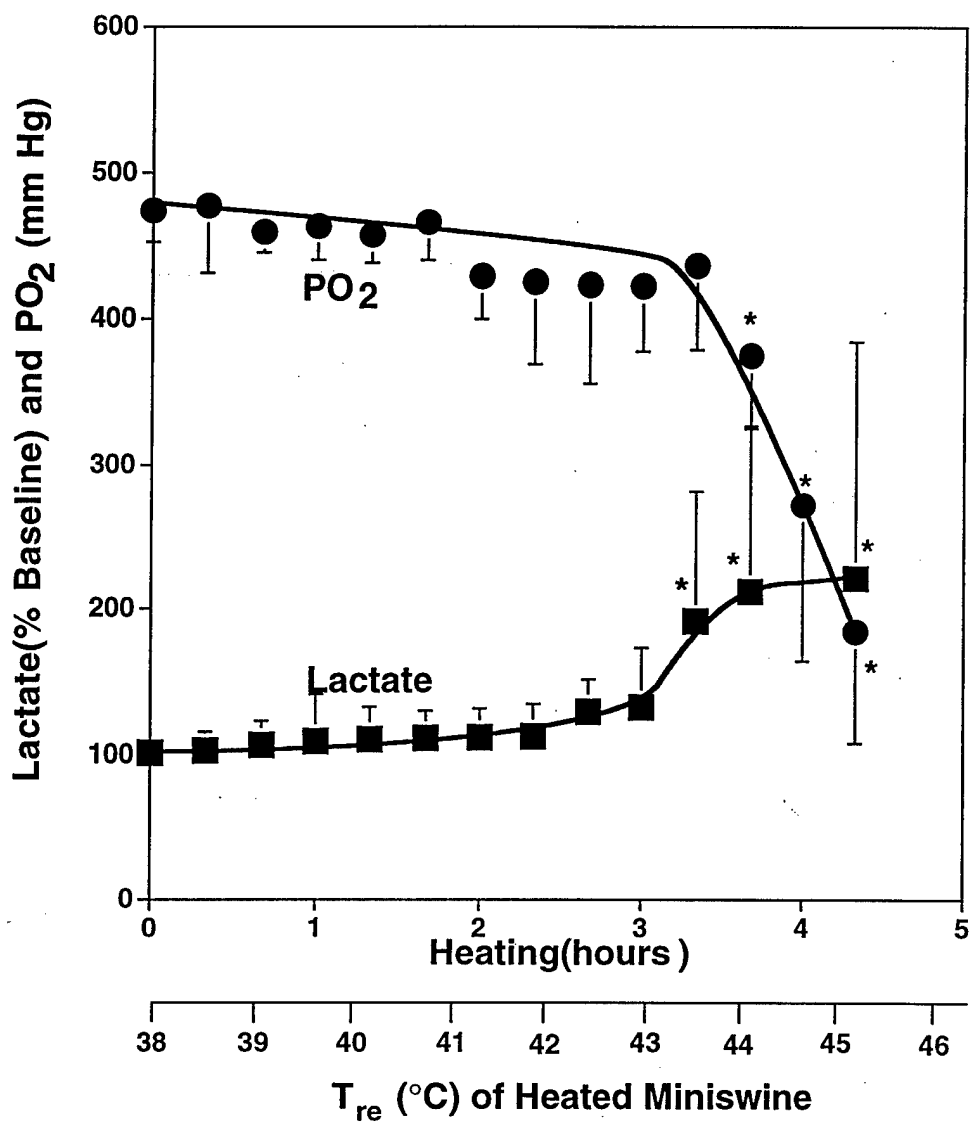


Fig. 2



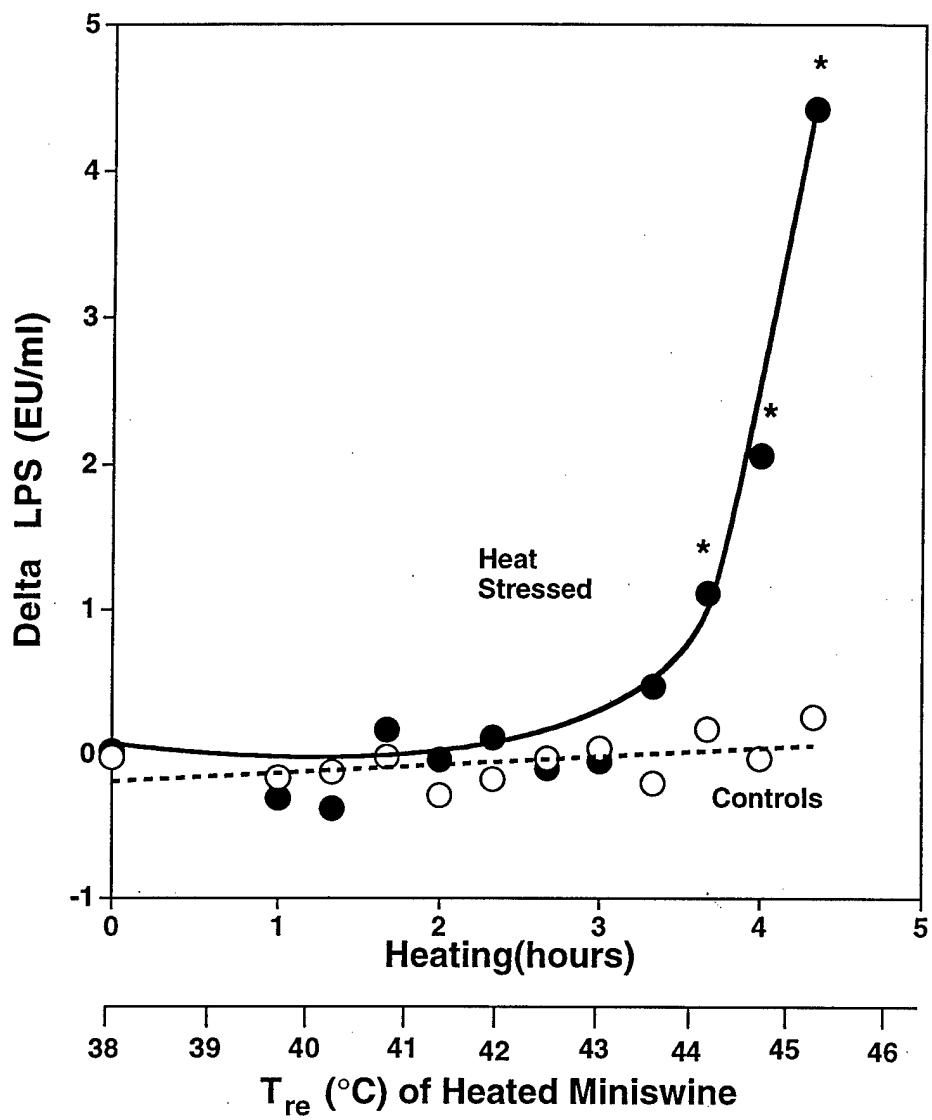


Fig. 4

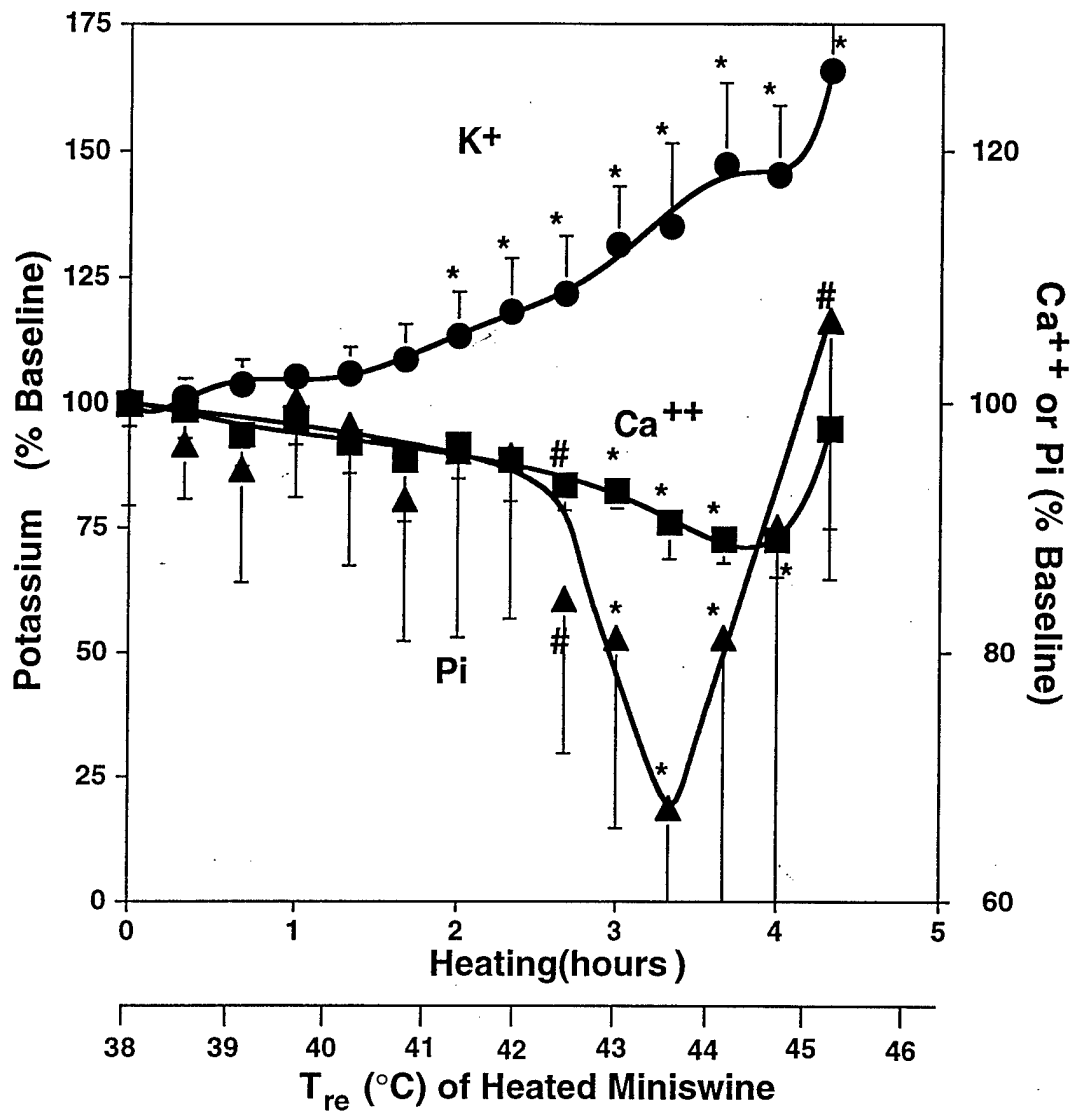


Fig. 5

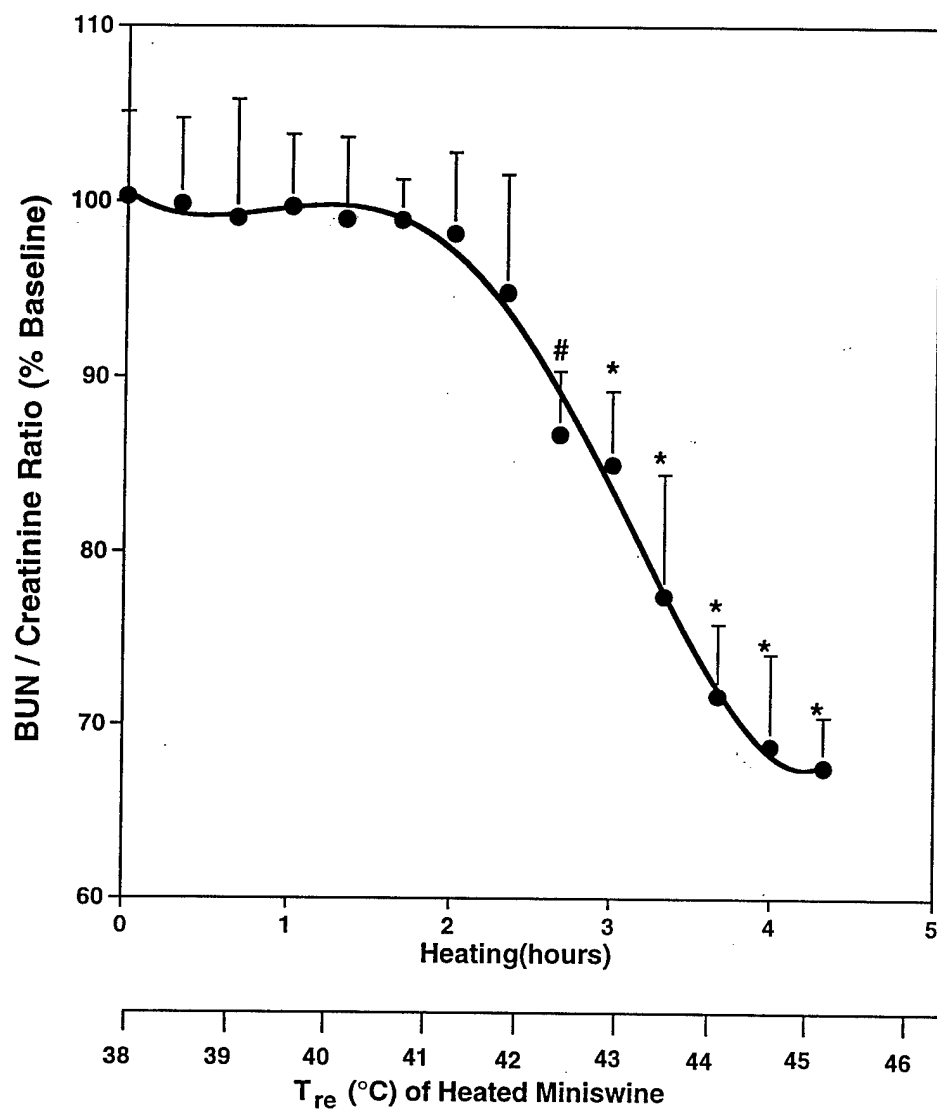


Fig. 6

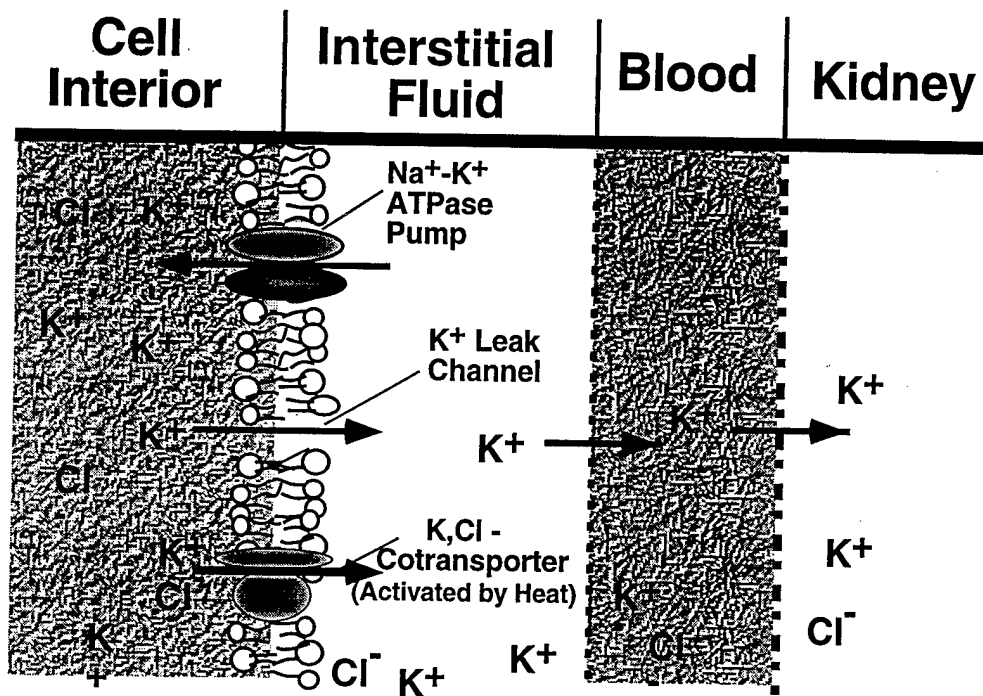


Fig. 7